HFA 3 or DMSP

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

January 19, 1999

Rec'd 1-22-99

Thomas B. Clarkson, D.V.M.
Professor of Comparative Medicine
Wake Forest University
School of Medicine
Medical Center Boulevard
Winston-Salem, North Carolina 27157-1040

Dear Tom:

Thank you for your kind words of congratulations. While I do miss the beauties of New Mexico, it is indeed an honor to be back at the Food and Drug Administration (FDA) as its Commissioner. It is good to hear from old friends and colleagues as I begin my tenure here at the Agency. I have fond memories of our work together on the NIH-NCRR council. I hope that along with your academic pursuits and research efforts, you still find time to enjoy raising ponies.

I appreciate your sharing with me concerns about the inclusion of alcohol-washed soy protein in the Agency's proposal to allow a coronary heart disease health claim for soy protein. I am sending a copy of your letter to our Center for Food Safety and Applied Nutrition to consider along with any other comments we receive on this health claim.

Thank you, as well, for your offer to help the Agency as we proceed on this rulemaking.

Sincerely,

Jane E. Henney, M.D.
Commissioner of Food and Drugs

98P-0683

LET 2

WAKE FOREST
UNIVERSITY
SCHOOL of MEDICINE
THE BOWMAN GRAY CAMPUS

December 29, 1998

Jane E. Henney, M.D.
Commissioner of Food & Drugs
Food & Drug Administration
5600 Fishers Lane HF-1
Rockville, MD 20857

Dear Jane:

Congratulations on your new appointment. No doubt it is good to get back to Washington, but I am sure you miss the beauty of New Mexico.

For the past half dozen years, we have been supported by a program project grant from the NHLBI to research the cardioprotective effects of soy protein and its components. A special focus of our research has been to investigate if, and to what extent, soy phytoestrogens (isoflavones) could be used as an alternative to traditional hormone replacement therapy for postmenopausal women. We were pleased to read in the *Federal Register* that the Food and Drug Administration had approved a coronary heart disease health claim for soy protein, but we were deeply concerned to read that the health claim would be extended to include alcohol-washed soy protein. Naturally, alcohol washing removes the phytoestrogens or isoflavones.

Attached is a letter sent to the Dockets Management Branch which reviews our evidence that the great majority of the cardioprotective effect of soy protein is removed by alcohol washing. I wanted to be sure that you realized the negative public health impact of extending the coronary heart disease health claim to alcohol-washed soy.

If I can be helpful to you and/or the FDA in considering the matter, please let me know.

With kindest personal regards,

Sincerely,

Tom

Thomas B. Clarkson, D.V.M.
Professor of Comparative Medicine

Attachment

Comparative Medicine Clinical Research Center
Medical Center Boulevard, Winston-Salem, NC 27157-1040 (336) 716-1500 / fax (336) 716-1515

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December 29, 1998

WAKE FOREST
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SCHOOL of MEDICINE
THE BOWMAN GRAY CAMPUS

Dockets Management Branch
HFA-305
Food and Drug Administration
"5630 Fishers Lane
Room 1061
Rockville, Maryland 20852

Re: Food Labeling: Health Claims; Soy Protein and Coronary Heart Disease (Dockets No. 98P-0683)

Attention Reviewers:

For the past several years our group has been supported by a Program Project Grant (HL45666) from the National Heart, Lung and Blood Institute to investigate the cardioprotective effects of soy protein. In the majority of our studies we have compared the effects of soy protein isolate that has been alcohol washed (referred to at our Center as Soy [-]) with non-alcohol washed soy protein isolate (referred to as Soy [+]). Our studies have utilized nonhuman primates as models (cynomolgus macaques) which share greater than a 90% homology with human beings in their DNA. In addition, the studies have extended to human beings by our colleague, John R. Crouse, M.D.

We agree completely with the FDA that a coronary heart disease health claim for soy protein is appropriate. We strongly agree with the FDA's conclusion that the above referenced health claim should be extended to alcohol washed soy protein isolate. All of our data indicate clearly that alcohol washing of the soy protein removes a majority of its cardioprotective benefits.

We have summarized below data from various studies that we believe supports our position that alcohol washed soy protein isolate has greatly attenuated benefits for cardiovascular disease relative to the natural (non-alcohol washed) soy protein isolate.

Rats and Hamsters

Recent studies have suggested that alcohol-extractable components of soy protein are largely responsible for the beneficial effects on plasma lipids and lipoproteins. Studies in rats and hamsters [Balmir et al. 1996] have shown lower LDL-C concentrations when they were fed a casein-based diet with an alcohol extract of soy protein added. Sugano and Koba [1993] found that a methanol-extract ed soy fraction was not as effective as the unextracted fraction in maintaining low plasma cholesterol concentrations in rats.

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Transgenic Mice

Transgenic mice lacking the LDL receptor were compared with C57BL/6 mice that had functional LDL receptors [Kirk et al. 1998]. Bothstrains ofmicewere fedanatherogenic diet that included either alcohol washed soy protein [Soy(-)] or non-alcohol washed soy protein [Soy(+)]. Only those mice with LDL receptors and fed Soy (+) had lowered plasma cholesterol concentrations and protection against atherosclerosis. Their findings are summarized in Figure 1. These data suggest that intact (non-alcohol washed) soy protein isolate is significantly better than the alcohol washed soy protein isolate for reducing plasma cholesterol concentrations and atherosclerosis.

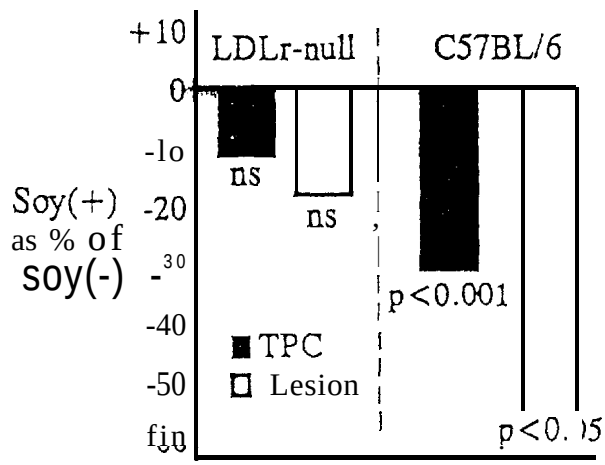

Figure 1. Effects of non-alcohol washed soy protein [Soy(+)] on TPC and atherosclerotic lesions in LDL receptor null (LDLr-null) and control mice (C57BL/6) expressed as a % of the Soy (-) effect [Kirk et al. 1998].

Modified from Kirk et al., J Nutr, 1998

Plasma Lipids/Lipoproteins of Male and Female Monkeys

Our own work strongly supports the conclusion that alcohol extractable components play a primary role in modulating soy protein's effects on plasma lipid and lipoprotein concentrations. In a cross-over study in young rhesus monkeys (n= 11 males, n= 14 females), moderately atherogenic diets containing alcohol washed or non-alcohol washed soy protein isolate were fed for 6 months [Anthony et al. 1996]. LDL+VLDL-C concentrations were significantly lower with the Soy(+) diet in both males and females. In the females, the Soy(+) diet resulted in significantly improved HDL-C concentrations relative to when they were fed the Soy(-) diet.

This finding prompted us to extend the findings to include atherosclerosis and to again compare alcohol washed [Soy(-)] and non-alcohol washed [Soy(+)] soy protein with casein and lactalbumin fed as a part of a moderately atherogenic diet. We randomized 85 male and 75 female cynomolgus monkeys into one of three treatment groups: 1) casein and lactalbumin as the source of protein (Casein), 2) the Soy(-) diet, or 3) the Soy(+) diet [Anthony et al. 1997 and 1998]. The diets were identical in composition for macronutrients. Effects of these diets on LDL-C and HDL-C are shown in Figure 2. Soy(-) and Soy(+) group means are expressed as the percent difference from the casein and lactalbumin group.

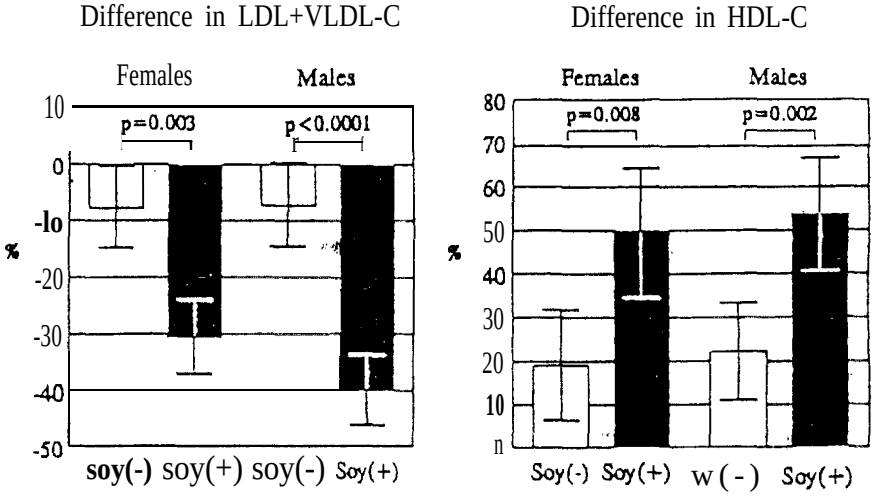

Figure 2. Effects of diets containing soy protein isolate washed with ethanol [Soy(-)] or non-alcohol washed soy protein [Soy(+)] on LDL+VLDL-C (left panel) and HDL-C (right panel) for female and male cynomolgus monkeys. The bars represent the percent improvement in lipoprotein concentrations compared to the Casein group. From Anthony et al. [1998].

Atherosclerosis of Male Monkeys

Two studies in young male nonhuman primates [Anthony et al. 1996, Anthony et al. 1997] have shown significant improvements in plasma lipids and lipoproteins when treated with non-alcohol washed soy protein [Soy(+)] as compared to alcohol washed soy [Soy(-)]). In the one study [Anthony et al. 1997] there was a further comparison to a group getting casein and lactalbumin as the dietary protein and atherosclerosis was measured, Data from this study are summarized in Figure 3.

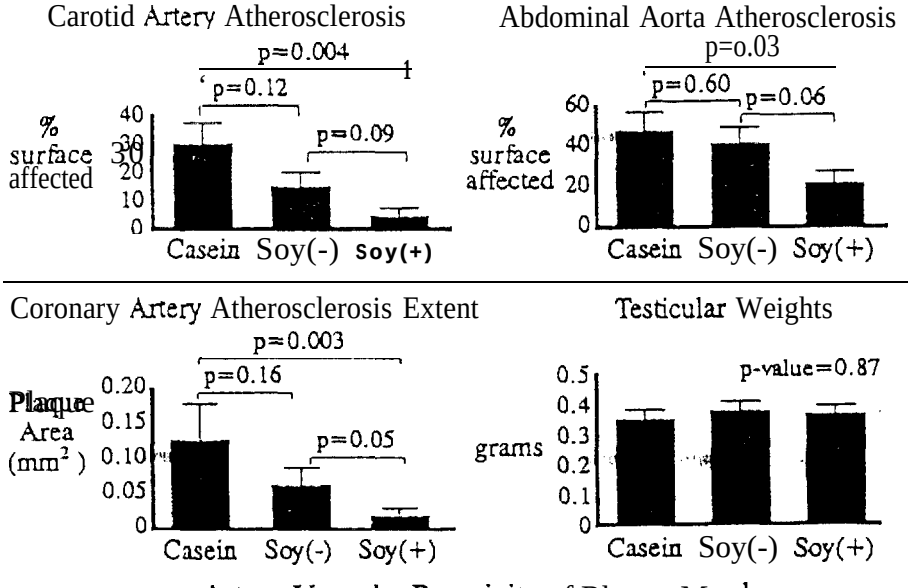

Figure 3. Effects after 14 months of treatment with soy (+) or soy (-) compared to animal protein-containing diets (casein) on atherosclerosis in carotid arteries, coronary arteries, and abdominal aorta (n=11/group). Data shown are adjusted means ± SEM. Adapted from Anthony et al. [1997].

onary Artery Vascular Reactivity of Rhesus Monkeys

The ability of coronary arteries to dilate rather than constrict when presented with an agonist for dilation (ie acetylcholine, serotonin) is an important aspect of cardioprotection. We have developed the capability of making such assessments *in vivo* in monkeys using quantitative angiography [Williams et al. 1994]. As a part of a study of rhesus monkeys with diet-induced atherosclerosis, we compared the effects of treatment with alcohol washed [Soy(-), n=6] vs non-

alcohol washed [Soy(+), n=5] soy protein on the capacity of coronary arteries to dilate in response to acetylcholine infusion [Honoré et al. 1997]. The results of that study are summarized schematically **(Figure** 4).

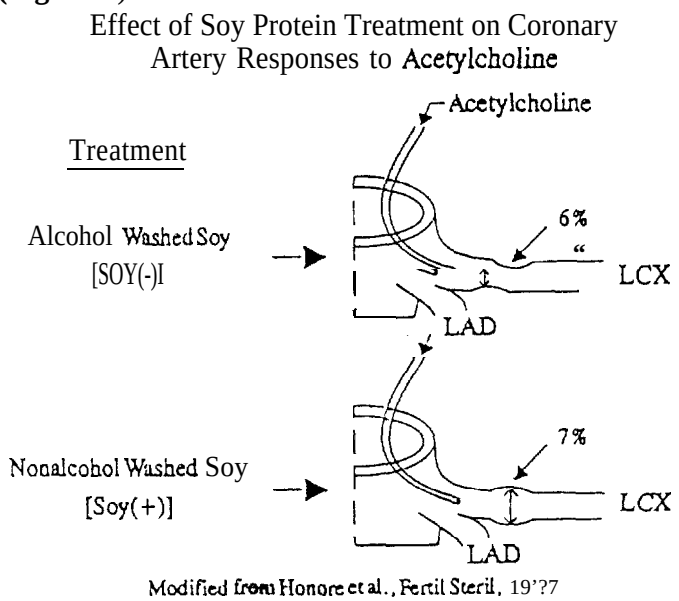

Figure 4. Coronary artery vascular **reactivity in response to intracoronary acetylcholine** administration in **rhesus** macaques **fed diets containing alcohol washed soy protein [Soy(-)] or non-alcohol washed soy protein [Soy(+)]. Adapted from Honoré et al [1997].**

Of considerable importance was the finding that coronary arteries constricted when the animals had been treated with Soy (-) but dilated when the animals had been treated with Soy (+).

Blood Flow Following Intra-Coronary Artery Platelet Aggregation

It is well known that feeding soy protein inhibits platelet activation. Recently, it was reported that alcohol soluble components of soy protein have the capacity to inhibit serotonin uptake by platelets [Helmeste & Tang 1995]. Atherosclerosis augments the constrictor responses of coronary arteries to serotonin. We hypothesized that feeding diets containing non-alcohol washed soy protein should inhibit platelet-mediated constriction of atherosclerotic arteries by virtue of having reduced the serotonin content of the platelets. Thus, we examined the effects of the soy protein diet (comparing alcohol washed vs non-alcohol washed soy protein) on constrictor responses of coronary arteries following platelet activation induced by intracoronary infusion of collagen [Williams and Clarkson, in press]. A summary of the results are shown in Figure 5.

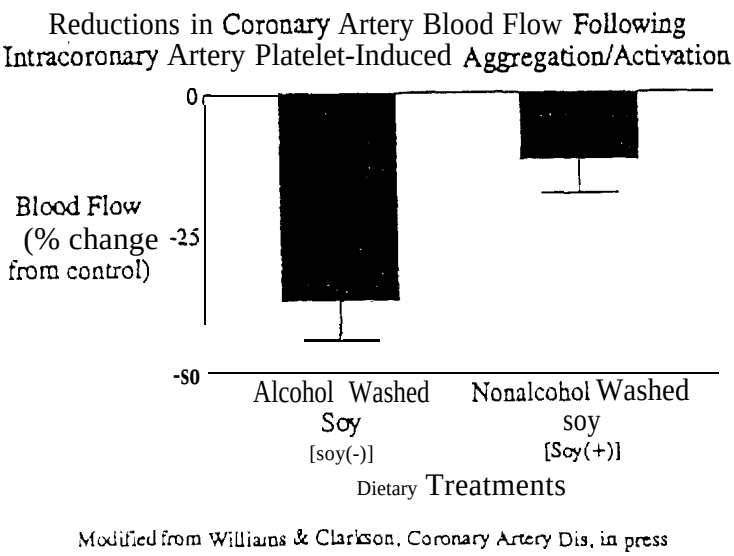

Figure 5. Change in coronary artery blood flow in response to intracoronary infusion of collagen in Soy (-) and Soy (+) treated monkeys. Results indicate that non-alcohol washed but not alcohol washed soy may protect against platelet-mediated decreases in coronary artery blood flow.

Intact (non-alcohol washed) dietary soy protein (but not alcohol washed soy protein) inhibited the reduction in coronary artery blood following collagen-induced platelet aggregation and serotonin release.

Plasma Lipids/Lipoproteins of Postmenopausal Female Monkeys

In Table 1 we have summarized the differences in plasma lipid/lipoprotein concentrations of surgically postmenopausal cynomolgus monkeys fed a moderately atherogenic diet for three years [Anthony & Clarkson 1998; Clarkson & Anthony 1998]. Both groups were fed soy protein as the primary source of protein in the diet, however, the soy peptide had either been alcohol washed [Soy(-)] or not [Soy(+)].

Table 1. Effects of Treatment with Alcohol Washed [Soy(-)] or Non-alcohol Washed [Soy(+)] Soy Protein on Cardiovascular Risk Factors of Surgically Postmenopausal Monkeys.

	Alcohol Washed **Soy (-)** n = 56	Non-alcohol Washed Soy (+) n =59	p - **value**
LDL + VLDL-C (mg/dl)	316 ± 13	251 ± 12	*0.0003*
HDL-C (mg/dl)	62 ± 2	72*2	*0.004*
Plasma triglycerides (mg/dl)	38 ± 3	35 ± 3	0.55
TPC:HDL-C	8.03 ● 0.64	5,49 ± 0.63	0.005
Apo A- 1 (mg/dl)	236 ± 9	271*9	0,005

Atherosclerosis of Postmenopausal Female Monkeys

We have begun to evaluate the effects of long-term (3 year) treatment with alcohol washed or non-alcohol washed soy protein isolate in postmenopausal monkeys. Two parts of those evaluations are now complete.

Part 1. We took advantage of the fact that atherosclerosis extent is nearly identical between the left and right common iliac artery (correlation coefficient = 0.97). The monkeys had been fed a diet mimicking that of human beings in fat and cholesterol for 2 years before interventions were begun in order to induce atherosclerosis to a stage comparable to that in 50 year old women. Immediately before initiating the treatments, one common iliac artery was removed as a record of the baseline amount of atherosclerosis for each animal. The baseline artery was then compared with the contralateral iliac artery after three years of treatment so that we could determine for each monkey whether her atherosclerosis progressed during treatment. The results of those evaluations are summarized as a part of **Table** 2.

Part 2. Because atherosclerosis of internal carotid arteries has an important bearing on stroke occurrence we have studied that artery in detail. Those data are also a part of **Table** 2.

"Table 2. **Effects** of Treatment **with Alcohol Washed [Soy(-)] or Non-alcohol Washed [Soy(+)] Soy Protein on Atherosclerosis of Surgically Postmenopausal Monkeys.**

	Alcohol Washed Soy (-) n = 56	Non-alcohol Washed Soy (+) n =59	p - value
Internal Carotid Atherosclerosis (mm^2)	0.038 -0.010, +0.013	0.012 -0.003, +0.004	0.005
Iliac Artery Atherosclerosis (% of group with plaque progression)	64%	44%	0.03

From the data presented in **Table 2,** it is clear that there are significant differences in atherosclerosis outcomes depending on whether the soy protein isolate has or has not been alcohol washed.

Human Patients

Confirmation of our monkey studies, all indicating that alcohol washing of the soy protein removes a large part of its cardioprotective benefits, has now been completed in a study by our colleague John Crouse and his coworkers [1998]. They treated moderately hypercholesterolemic individuals (n= 150) with a 25 gram protein supplement given as a daily beverage, For one month prior to treatment, participants were counseled to eat a low-fat, low-cholesterol diet (National Cholesterol Education Program Step 1 diet). Baseline plasma lipids were then measured and they were randomly assigned to one of the supplement groups for nine weeks. The supplements were casein, alcohol washed soy protein isolate [Soy(-)], or non-alcohol washed soy protein isolates that contained 27, 37, or 62 mg isoflavones/day. There were 94 men and 62 women enrolled in this study. Plasma LDL-C concentrations were not significantly lower when treated with alcohol washed soy compared to the casein supplemented group. Plasma LDL-C concentrations were lower in the non-alcohol washed soy supplement groups containing at least 37 mg of isoflavones compared to the casein group, but the difference was only significant when the isoflavone intake was 62 mg/day. In the half of the group with higher baseline LDL-C concentrations, both the 37 and 62 mg/day supplements resulted in significantly lower LDL-C compared to the casein group. Importantly, there was a significant trend toward lower LDL-C concentrations with increasing isoflavone dose **(Figure 6). In** the whole group the p-value for trend did not reach statistical significance (p=0. 10), but in the high risk group (the upper half of the group for baseline LDL-C) the trend was significant for both LDL and TPC ($p < 0.025$).

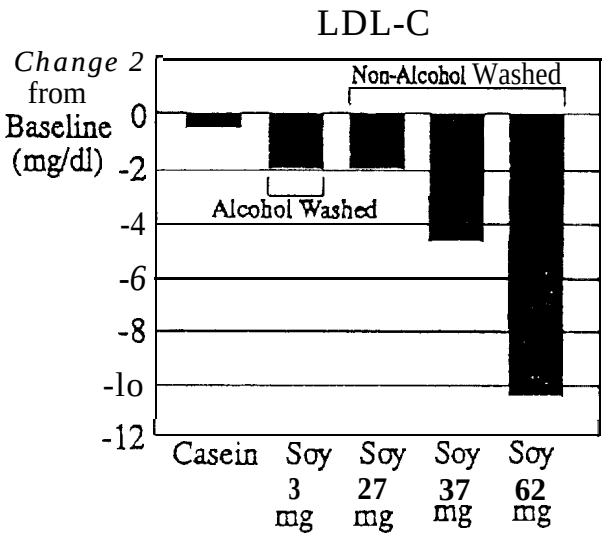

Figure 6. Effects on LDL-C of men and women given protein supplements: casein or alcohol washed vs non-alcohol washed soy protein with varying concentrations of isoflavones. Data are expressed as change from baseline (mg/dl).

Summary

When all these findings are considered together (our data in monkeys and the report by Crouse) there is a broad base of experimental and clinical evidence that alcohol washing of soy protein isolate removes the great majority of its cardioprotective potential.

We recommend that a coronary heart disease health claim be granted for ~~non-alto~~hol washed soy protein only.

Sincerely,

Mary S. Anthony, M.S.
Research Assistant

Sincerely,

Thomas B. Clarkson, D.V.M.
Professor of Comparative Medicine

TBC/mjb

References

Anthony MS, Clarkson TB, Hughes CL Jr, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr 1996; 126:43-50.

Anthony MS, Clarkson TB, Bullock BC, Wagner JD. Soy protein versus soy phytoestrogens in the prevention of diet-induced coronary artery atherosclerosis of male cynomolgus monkeys. Arterioscler Thromb Vase Biol 1997; 17:2524-2531.

Anthony MS, ClarkSon TB. Comparison of soy phytoestrogens and conjugated equine estrogens on atherosclerosis progression in postmenopausal monkeys. Circulation 1998;97: 829 (abstract).

Anthony MS, Clarkson TB, Williams JK. Effect of soy isoflavones on atherosclerosis potential mechanisms. Am J Clin Nutr 1998;68(suppl): 13905-13935.

Balmir F, Staack R, Jeffrey E, Berber Jimenez MD, Wang L, Potter SM. An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters. J Nutr 1996; 126:3046-3053.

Clarkson TB, Anthony MS. Effect of hormone replacement therapy on internal carotid artery atherosclerosis of postmenopausal monkeys. Circulation 1998;97: 830 (abstract).

Crouse JR, Terry JG, Morgan TM, McGill BL, Davis DH, King T, Ellis JE, Burke GL. Soy protein containing isoflavones reduces plasma concentrations of lipids and lipoproteins. Circulation 1998;97: 816 (abstract),

Helmeste DM, Tang SW. Tyrosine kinase inhibitors regulate serotonin uptake in platelets. Eur J Pharacol 1995;280:R5-R7.

Honoré EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril 1997;67: 148-154.

Kirk EA, Sutherland P, Wang SA, Chait A, LeBoeuf RC. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. J Nutr 1998; 128:954-959.

Sugano M, Koba K, Dietary protein and lipid metabolism: a multifunctional effect. Ann NY Acad Sci 1993 ;676:215-22.

Williams JK, Honoré EK, Washburn SA, Clarkson TB. Effects of hormone replacement therapy on reactivity in, atherosclerotic coronary arteries in cynomolgus monkeys. J Am Coil Cardiol 1994;24:1757-1761.

Williams JK, Clarkson TB. Dietary soy isoflavones inhibit *in vivo* constrictor responses of coronary arteries to collagen-induced platelet activation. Coronary Artery Dis, *in press*.